GATING CURRENT AND POTASSIUM CHANNELS IN THE GIANT AXON OF THE SQUID

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ABSTRACT Gating current (I_g) underlying Na-channel activation is large enough to enable resolution of components both preceding and paralleling Na conductance (g_{Na}) turn-on. For large depolarizations (beyond +20 mV), an additional "slow phase" of I_g is observed during a time when Na activation is already complete, but when K-channel opening is just becoming detectable. If Na- and K-channel gating are similar, the slow kinetics and long delay for K activation predict that K channel I_g must be relatively small and slow. Externally applied dibucaine almost totally blocks g_{Na} and greatly reduces the fast (Na channel) I_g without altering g_K or the I_g slow phase. The slow phase of I_g depends in part on the presence of functional K channels. Selective diminution in amplitude of the slow phase is consistently observed after a 30-min perfusion with both external and internal K-free media, a procedure which destroys nearly all K channels. This decrease of I_g amounts to ~10% of the total charge movement at +40 to +80 mV, with gating charge and K channels disappearing in a ratio of <1 e^- per picosiemens of g_K. These findings are consistent with the idea that part of the I_g slow phase represents gating current generated by the early steps in K-channel activation.

INTRODUCTION

Charged or dipolar "gating" molecules give rise to the voltage dependence of membrane capacitance in nerve. Intramembranous movement or reorientation of these charges regulates activation of Na channels and generates a capacitive gating current (I_g) after a voltage step (for a recent review, see Almers, 1978). A qualitatively similar mechanism may subserve voltage-dependent K-channel gating, but K-gating current has so far escaped detection. We describe here voltage-clamp experiments on perfused axons of *Loligo pealei* in which we identify a slow component of I_g as gating current that may be associated with K-channel activation.

METHODS

Standard methods were used for measuring I_g (Bezanilla and Armstrong, 1977). Control pulses were taken with the P/4 procedure from a level of −140 or −150 mV. Usually 40 or 80 sweeps were averaged, and the resultant was sometimes digitally filtered with a 15-μs time constant. Flat baselines were fitted during the final 1–2 ms of 6–7 ms pulses. Traces with visible baseline slope were deemed unreliable and were discarded. Our procedure (see below) required stable and long-lived axons; experiments in which peak I_g amplitude changed by >10% over the period of study were also discarded.

Internal solutions contained either 275 mM K or 200 mM tetramethylammonium (0 mM K). The anions were 50 mM F^- and 150–225 mM glutamate; isotonicity was maintained with sucrose. The external perfusion normally contained 0 mM K or 20 mM K plus 40–60 mM Cs, the Cs serving to block inward I_K (Adelman and French, 1978; see also below). Isotonicity was maintained with Tris; the anion

was Cl^-. I_g was measured in the presence of 10^{-7} M tetrodotoxin, (TTX), and equimolar substitution of 116 mM Na for Tris enabled I_{Na} measurements. All solutions were 950–1050 mosm and pH 7.0–7.3. Perfusion media are given below as external//internal, e.g., 20 K 60 Cs//0 K.

RESULTS

The Slow Component of Gating Current

For a depolarization beyond +20 mV, I_g shows a prominent slow phase, and its time-course is compared with I_{Na} and I_K in Fig. 1. A substantial amount of gating charge is moving well after I_{Na} has peaked and thus seems too slow to be associated with the opening of Na channels. In contrast, very few K channels are open before I_g has returned almost to the baseline. What we here refer to as the slow component of I_g represents charge movement during a time in which most Na channels are open and most K channels are still closed.

The slow component is not affected by internal pronase treatment, which destroys Na inactivation, and is thought not to reflect movement of a charged inactivation gating particle (Armstrong and Bezanilla, 1977). Two additional sources for the slow phase were suggested by these authors, one being a transition of open Na channels to a second open state, and the other being K gating current. Convincing evidence has been lacking to support either suggestion (Armstrong, 1978; Almers and Armstrong, 1980).

Dibucaine Blocks g_{Na} and Fast I_g, but does not Alter g_K or Slow I_g

Effects of the local anesthetic dibucaine do not readily support the Na-channel transition hypothesis and suggest K gating as the more likely source of at least part of the slow component. Externally applied dibucaine (0.2–0.5 mM) nearly totally blocks I_{Na} (Fig. 2 *A*), and greatly reduces the early, Na-channel related portion of I_g (Fig. 2 *B*). The drug does not markedly change the slow component of I_g, and parallel with this, it has little effect on g_K.

The effect of dibucaine on I_g and its lack of effect on g_K can be seen in the same traces in Figs. 2 *C* and *D*. In Fig. 2 *C* current was recorded in the absence of dibucaine during and after a large pulse of varying duration in 25 K//0 K (no Cs). I_g alone is seen during the pulse, as inward I_K is immeasurably small at this voltage. During the briefest pulse (0.3 ms) few K channels opened, and the tail current at pulse end is mainly inward I_g associated with Na channel closing. With increasing duration g_K progressively activates, and after 2 ms the tails

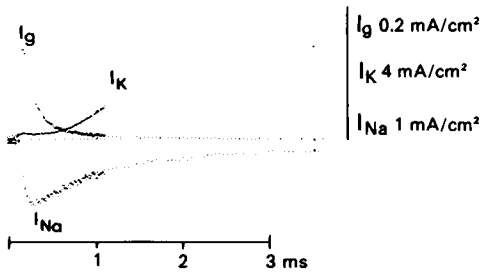

FIGURE 1 Comparison of time-courses of gating (I_g), Na (I_{Na}), and K (I_K) currents in the same axon at +60 mV. I_K was recorded after TTX addition in 20 K 40 Cs//275 K; I_g was recorded after washing out internal K; and I_{Na} was recorded after washing out TTX. The holding potential was −80 mV at 8°C. JN199A,B.

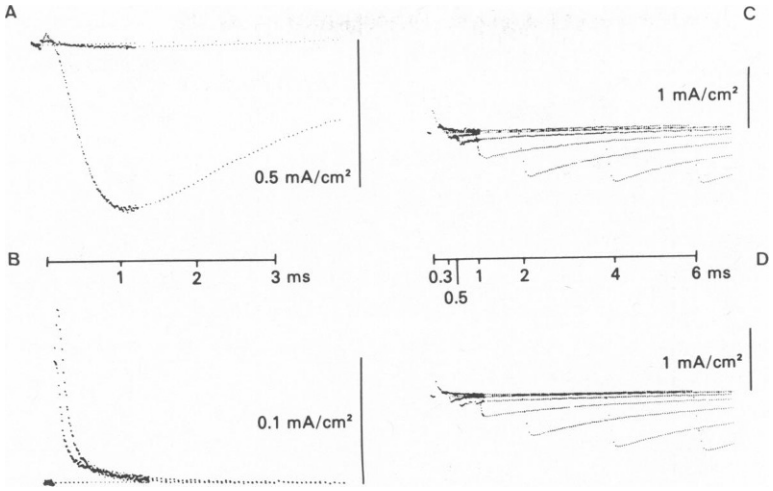

FIGURE 2 Action of externally applied dibucaine on I_{Na}, I_g, and I_K. (*A*) I_{Na} at 0 mV before (larger trace) and during 0.5 mM dibucaine. The holding potential was −70 mV at 8°C. MA159B. 0 K//0 K.(*B*) I_g at +50 mV before (larger trace) and during 0.2 mM dibucaine. The holding potential was −60 mV at 8°C. JN229A. 20 K 60 Cs//0 K. (*C*) Family of currents before dibucaine at varying pulse durations (see text for details). ON current is I_g; OFF current is $I_g + I_K$. The holding potential was −70 mV at 8°C. MA249C. 25 K//0 K. (*D*) Currents corresponding to *C* during 0.25 mM dibucaine. Same axon as *C*.

are much larger and composed mainly of I_K. The envelope of peak tail current amplitudes gives the approximate time-course of K-channel activation.

Tail currents in dibucaine are similar (Fig. 2 *D*), and the predominately I_K tails for pulses of >2 ms are not affected by the drug. The 0.5- and 1-ms tails are somewhat reduced, suggesting that dibucaine may slow K-channel opening, but the effect is slight.

Dibucaine thus blocks Na channels and reduces the early part of I_g. It has little effect of K-channel activation or on the slow phase of I_g, consistent with the idea that the slow phase is related to K-channel gating.

The Slow Phase of I_g is Diminished when Functional K Channels are Lost

Chandler and Meves (1970) first noted the apparent destruction of g_K after internal perfusion with a K-free solution. More recently, Almers and Armstrong (1980) have studied this phenomenon in detail. They found, and we confirm, that bathing an axon for 30 min in K-free media inside and outside (0 K//0 K) results in irreversible loss of >90% of functional K channels. The channels function quite normally and are preserved from destruction if as little as 20 mM K is added to the external solution (e.g., Fig. 2 *C*).

We have searched for a change in I_g accompanying loss of functioning K channels. The strategy was to measure I_g in low K//0 K when the channels were functional, then to destroy g_K by perfusing 0 K//0 K for 30 min, and finally to repeat the I_g measurement in low K//0 K (c.f. Almers and Armstrong, 1980).

In practice, the low K external solution also contained Cs in the ratio 2 or 3 Cs:1 K. The Cs totally blocked inward I_K during the pulses without interfering with K-channel gating. Briefly, the evidence is (*a*) the instantaneous *I-V* relation in 40 K 80 Cs//0 K is not detectably

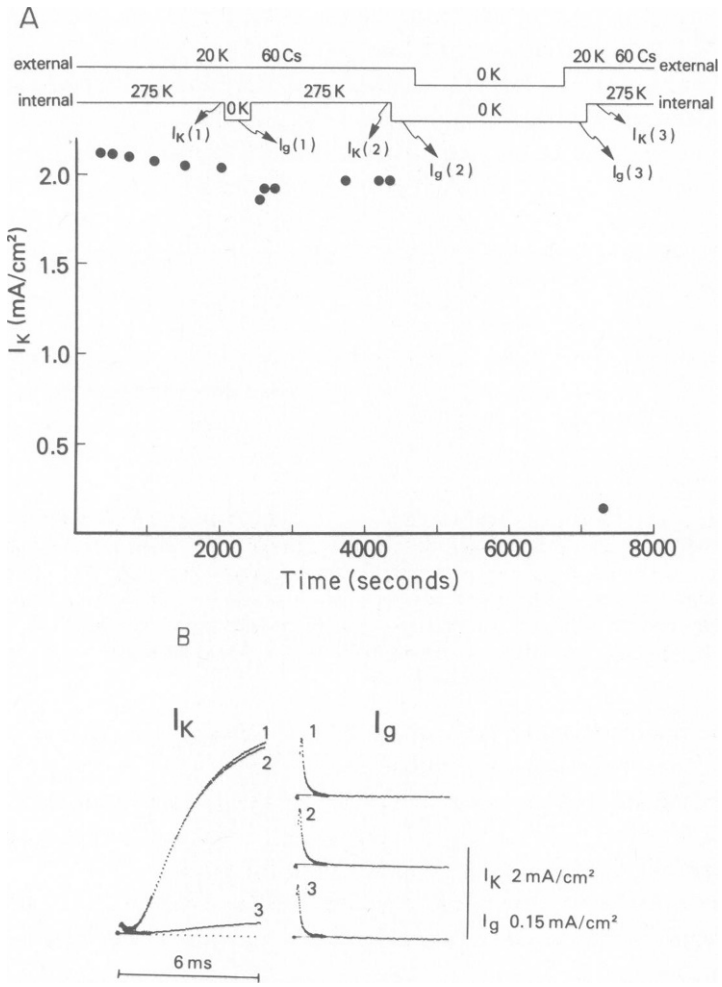

FIGURE 3 Protocol and results from a 0 K//0 K K channel washout experiment. (*A*) Progression of external and internal solution changes during the experiment is indicated at top. Arrows indicate times the various records in *B* were taken. Points plotted are I_K at +60 mV as a function of time. Note the large decrease in I_K caused by the 0 K//0 K perfusion. (*B*) I_K and I_g records taken at times indicated in *A*. Records at times (1) and (2) should be compared for the control soak, while comparison of the times (2) and (3) traces shows the changes due to prolonged 0 K//0 K perfusion. The holding potential was −80 mV at 8°C. JN309B.

different from that in 0 K//0 K, and (*b*) the kinetics and amplitude of outward I_K in 20 K 60 Cs//275 K are not detectably different from that in 20 K//275 K.

Fig. 3 *A* shows the progression of solutions, the times at which various I_K and I_g records in Fig. 3 *B* were taken (arrows), and the time-course of I_K decrease (plotted points) during a 0 K//0 K experiment. $I_K(1)$ was recorded after 2,000 s of soaking in 20 K 60 Cs//275 K (Fig. 3 *B*). Internal K was then washed out, and $I_g(1)$ was measured. Internal K was quickly reintroduced, and perfusion with this solution continued for 30 min. During this control soak there was little change in I_K [compare $I_K(1)$ and $I_K(2)$ in Fig. 3 *B*] and no detectable change in I_g [$I_g(1)$ vs. $I_g(2)$].

After recording $I_g(2)$ the fiber was bathed in 0 K//0 K for 30 min to destroy g_K. $I_g(3)$ was recorded in 20 K 60 Cs//0 K at the end of this period and represents I_g after most K channels were no longer functional. The loss of K channels was ascertained by record $I_K(3)$, which was taken shortly afterward upon restoring the internal 275 K.

$I_g(2)$ and $I_g(3)$, recorded before and after the 0 K//0 K soak, respectively, are superimposed in Fig. 4 *A*. They clearly differ, most noticeably in the amplitude of the slow phase, which is decreased about twofold. Fig. 4 *B* shows the difference $I_g(1) - I_g(3)$ at higher gain. The difference current, referred to as ΔI_g, is the component of I_g that disappears together with functioning K channels and constitutes ~10% of the total charge movement. We suggest that ΔI_g is associated with K-channel gating.

In an attempt to account for inevitable fiber rundown during the 0 K//0 K soak, we also obtained ΔI_g after scaling $I_g(3)$ by a factor of 1.09 to match its peak with that of $I_g(2)$. ΔI_g is not drastically changed by this scaling procedure, as can be seen by comparing the upper (no scaling) and lower (after scaling) traces in Fig. 4 *B*.

Fig. 4 *C* shows that the control soak caused no change in I_g. I_g (1) and $I_g(2)$ are

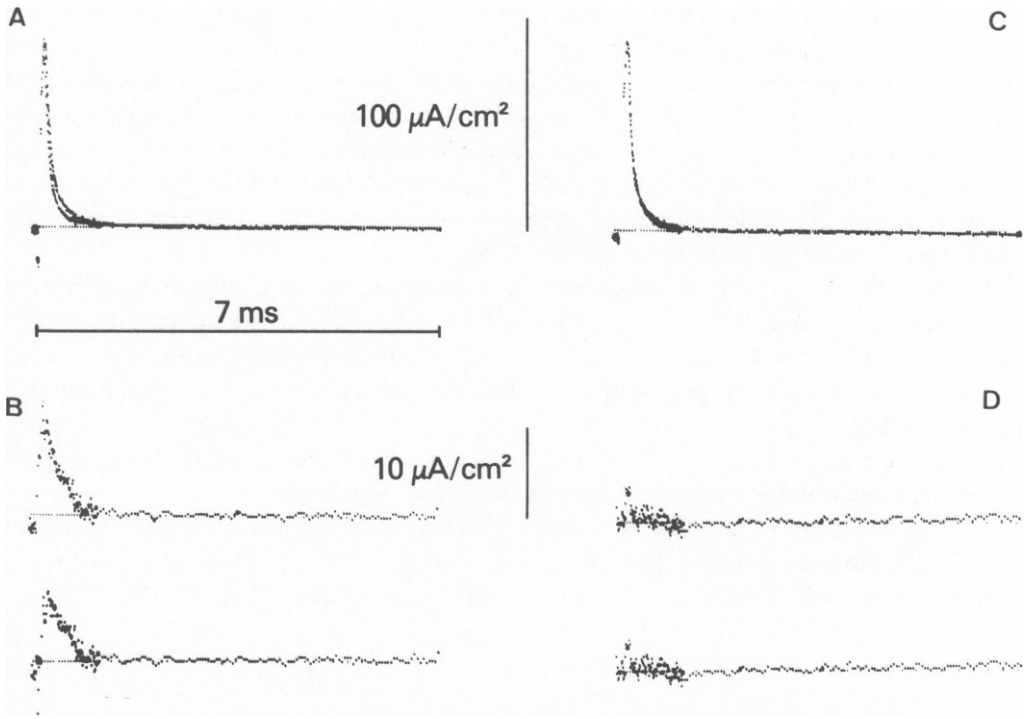

FIGURE 4 Comparison of I_g traces and ΔI_g with and without functional K channels present, and the difference current associated with K gating. Same axon as Fig. 3. (*A*) Traces before and after the 0 K//0 K soak are superimposed [$I_g(2)$ and $I_g(3)$ from Fig. 3 *B*]. A decrease in amplitude of the slow phase following K-channel loss is clearly visible. (*B*) Upper trace: difference between the two traces in *A* at higher gain. Lower trace: difference current after scaling $I_g(3)$ by 1.09 to account for fiber rundown. (*C*) Superimposition of I_g traces both taken during the control period [$I_g(1)$ and (2) from Fig. 3 *B*]. No difference is visible. (*D*) Upper trace: difference between traces in *C*. Lower trace: difference after scaling $I_g(2)$ by 0.99.

superimposed, and they are not detectably different. Fig. 4 *D* gives the difference between $I_g(1)$ and $I_g(2)$ at higher gain, both without (upper trace) and with (lower trace) scaling of $I_g(2)$ to match its peak to $I_g(1)$.

Other tests discounted several artifactual sources of the ΔI_g ascribed above to K-channel loss. Subtraction of I_g traces taken at slightly different voltages indicated that an error in voltage measurement (e.g., from electrode drift) would have had to be >10 mV to generate a ΔI_g comparable to that observed. This is far more error than expected on the basis of electorde drift, which was checked at the end of each experiment. Moreover, ΔI_g after 0 K//0 K was always as shown in Fig. 4 *B* and was never inward or totally absent, as would be expected from random electrode drift.

A voltage error invisible to the electrodes would likewise have had to be equivalent to >10 mV of membrane potential. Such an error might result from an irreversible change in surface charge density in the immediate vicinity of Na channels due to 0 K//0 K perfusion. In three axons studied, the $g_{Na} - V$ relation was shifted by <5 mV after 0 K//0 K treatment.

DISCUSSION

I_K and ΔI_g in the same axon are compared in Fig. 5. ΔI_g has decayed almost completely before I_K has much risen. By analogy with Na-channel activation, which has several fast steps followed by a slower one (Armstrong and Gilly, 1979), we think that ΔI_g is associated with early, fast steps in the activation of K channels. A still slower component associated with K-channel gating, having the time-course of dg_K/dt, is implied by this hypothesis, but has not yet been identified. A "dg/dt component" of gating current has been postulated for K channels in skeletal muscle (Adrian and Peres, 1977) and can, in fact, be clearly resolved for Na channels in squid (Armstrong and Gilly, 1979).

A final question concerns the amount of charge movement lost in relation to the number of K channels destroyed by 0 K//0 K perfusion. Amounts of g_K lost and the charge (ΔQ) carried by ΔI_g from five axons are given in Table I. The last column expresses these figures as the number of electronic charges per picosiemens of g_K. The average value is 1.3 e^-/pS. Assuming a single K-channel conductance of ~10 pS (Conti et al., 1975), this yields 13 e^-/channel, somewhat more than the minimum value of 5–6 e^- expected from the *e*-fold change in g_K in 4–5 mV (Hodgkin and Huxley, 1952; Almers and Armstrong, 1980).

The estimate of 13 e^-/channel is probably high for two main reasons. (*a*) ΔQ values in Table I were obtained without scaling of ΔI_g traces to account for rundown. Scaling typically reduces ΔQ by ~30%. (*b*) The amount of g_K ($\Delta I/\Delta V$ at pulse end in 20 K 40–60 Cs//275 K)

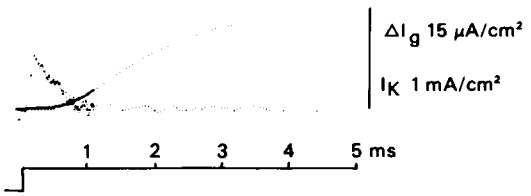

FIGURE 5 Comparison of I_K and its proposed gating current in the same axon. ΔI_g is the lower trace from Fig. 4 *B*. I_K was recorded at the beginning of the experiment in 20 K 60 Cs//7275 K; I_g has been subtracted out.

TABLE I
LOSS OF CHARGE MOVEMENT AND K CHANNELS

Axon	V_m	g_K lost	ΔQ lost	Lost
	(mV)	*(mS/cm²)*	*(e^-/μ^2)*	*(e^-/pS)*
AU319D	+60	24.3	165	0.7
	+70	25.8	118	0.5
JN309B	+60	13.1	269	2.0
	+50	—	245	—
JN299A	+80	9.6	183	1.9
JN209A	+60	21.4	412	1.9
	+80	24.8	240	1.0
JN199A*	+60	~18	140–330	0.8–1.8
	+80	~20	140–250	0.7–1.3

*No I_K record taken after 0 K//0 K soak; 10% assumed to remain. Range in ΔQ from difficulty in fitting a baseline due to a slow pedestal lasting ~2 ms.

Amount of g_K lost by the 0 K//0 K perfusion and the change in I_g (ΔQ) as a result. g_K was taken as the instantaneous $\Delta I/\Delta V$ at the end of a 6–7-ms pulse. ΔQ was obtained by integrating I_g difference currents (ΔI_g) after fitting baselines between 2 and 3 ms and digitally filtering.

lost is underestimated by ~20% since external Cs blocks the inward I_K tail. Taking these factors into account, a better estimate would be <10 e^-/channel. Though still more than expected, the agreement within a factor of 2 is encouraging in view of the difficulties in the procedure.

Insensitivity of the slow component of gating current to dibucaine, and its dependence on functional K channels, suggests that this slow charge movement is associated with K-channel gating. By analogy with the much faster and more easily resolved Na-channel gating current, we feel the time-course and amplitude of ΔI_g are consistent with the requirements for K-channel gating current. The role of the slow charge movement remaining after K-channel destruction (Fig. 4 A) remains unknown.

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